Studies of Fertilization in the Dog-Salmon, *Oncorhynchus keta*

1. The Morphology of the Normal Fertilization[1)]

By

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(With 10 Text-figures and 2 Plates)

Because of the fundamental importance in connection with researches in various fields of biology, the attraction of earlier workers has been concentrated on fertilization phenomena in animal eggs, from both morphological and physiological standpoints. Thus one can now find an enormous number of works contributing to this field of study in invertebrates as well as vertebrates.

With reference to the literature (cf. Makino's list, 1951), several earlier papers are to be accessible dealing with the fertilization phenomena of the fish egg. Kupfer (1886) seems to be the author who firstly undertook the morphological study on the fertilization in the egg of the trout. Then the studies along this line were followed by Boem (1891), Blanc (1891) and Behrens (1898) in eggs of various salmonid fishes. Quite recently, Ozima (1943) has published a cytological study concerning the maturation and fertilization of the egg of the carp (*Cyprinus carpio*). Viewed from the present status of knowledge especially furnished recently by many physiological studies in this field, however, important problems on the morphological aspect of fertilization have been remained not sufficiently defined in fishes, particularly in the Salmonidae.

In a series of studies to be continuously published hereafter, the author will deal with the physiology of fertilization phenomena in *Oncorhynchus keta*, especially from the histochemical point of view. Previous to inquiry into various physiological events of fertilization, it is necessary to get an accurate understanding of morphological phenomena. With this view in mind, the present investigation has been undertaken to make clear cytologically the course of the maturation and fertilization in the egg of the dog-salmon, with special attention towards the

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behavior of the spermatozoon after insemination.

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Material and Method

This study has exclusively carried out with the dog-salmon, *Oncorhynchus keta*, which was fished at the Chitose and the Oboro hatcheries in Hokkaido. The ripe eggs taken from a single mother fish were artificially fertilized by dry method. Then the eggs were allowed to develop in water at temperatures ranging from 7.5° to 9°C. A certain number of eggs were taken out at required intervals for fixation. As the fixatives, Bouin's solution and Bouin-Allen's solution were employed. The eggs obtained in 1948 and 1950 provided the material for study. Sections were prepared by paraffin method ; previous to imbedding the material, the chorion and superflous yolk were removed from the egg. The sections of 10 micra thick, stained with Delafield's haematoxylin and Heidenhain's iron-haematoxylin with counter-staining of eosin proved satisfactory for the purpose of the present study.

Descriptive

1). *The spermatozoon*

The spermatozoon closely resembles in external feature that of the salmon described by Bullowitz.[1] It has a simplest form ; a spheroidal head, a short and round middle piece, and a very long tail having no supplemental filament. The tail appears to attach to the margin of the middle piece.

2). *The ovum*

The ripe egg is remarkably large in size, usually 6 mm in diameter ; it is nearly spherical in form and moderately red colored. The egg-envelope is not transparent and very thick, so that the egg body can not be externally discernible. The envelope consists of two layers, an outer hyaline layer[2] and an inner membrane. The hyaline layer is transparent measuring some 20μ, when the egg is immersed in water. The inner membrane underneath the hyaline layer is thick and not transparent. It can be subdivided again into two parts. The micropyle is found penetrating the envelopes at the animal pole. Beneath the membrane the egg proper directly lies leaving no perivitelline space. The oöplasm enclosing the yolk

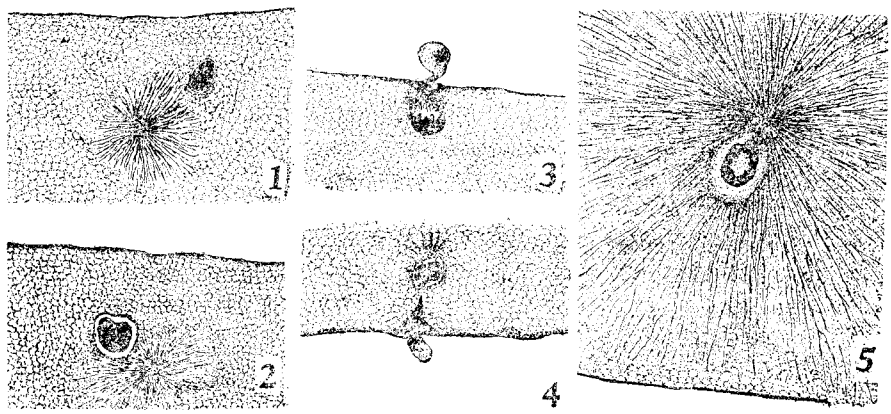
1) By reference to Wilson (1928).

2) This layer was observed by Aoki and named "Tōmeiso". Kagaku, vol. 16 ,1941.

is fairly thick at the animal pole and becomes thinner passing to the vegetable pole. The cortical alveoli can readily detected in section being embedded in the cortical layer. The fat globules of various sizes are present in the yolk. At the time of fertilization the egg nucleus shows the metaphase spindle of the second polar division which lies near the periphery of the animal pole with radial or slightly oblique direction. Generally the spindle lies slightly apart the middle part of the animal pole (Fig. 1).

3) *The formation of the male and female pronuclei*

The present study failed to follow the actual feature regarding the entry of the spermatozoon in sections. The section of the egg observed at 10 minutes after insemination showed the break-down of the cortical alveoli and the penetration of the spermatozoon into the egg (Fig. 2). There is a slight depression in the surface of the egg where the spermatozoon has entered. Apparently the entrance-area is cone-shaped and the apex of the cone points inwardly. Its outer and central portions are cross-straited in appearance. The perforation appears as a conspicious pore lying along the central axis of the cone and the spermatozoon is found lying near the apex of the cone. The figure here observed highly resembles the "entrance cone" observed by King (1901) in *Bufo* and also the "pseudo-micropyle" reported by Smith (1912) in *Cryptobranchus*. No sperm-tail could be detected in sections. Also the middle piece was difficult to clearly follow its history during metamorphosis in the present material, while Blanc (1894), in

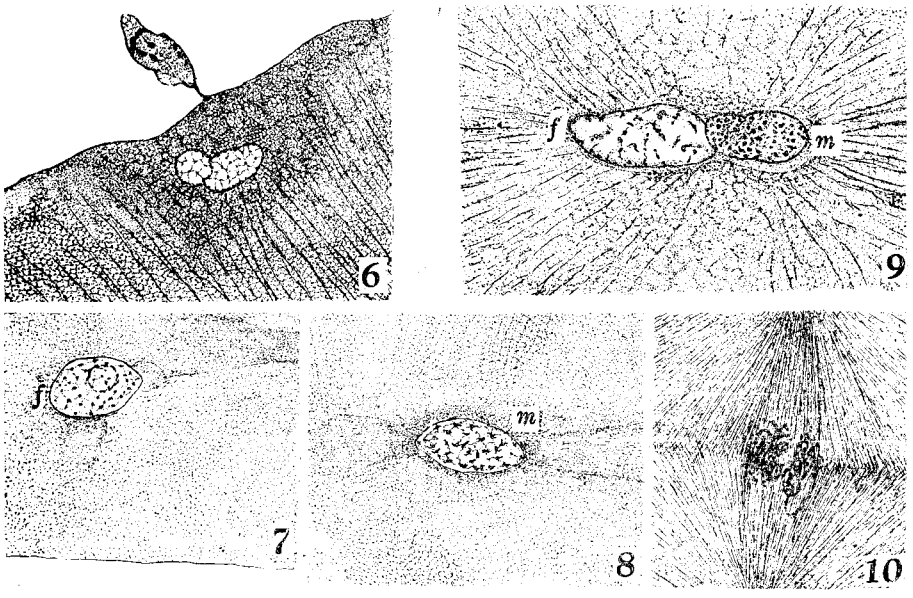


Text-fig. 1. Sperm-aster developed at the base of the sperm-head. From an egg about one hour after insemination. **Text-figs. 2-3.** Sperm-head with its aster and polar spindle in process of formation of the polar body. At two hours after insemination. **Text-figs. 4-5.** Chromosome mass and sperm-nucleus of vesicular form. From an egg about two and a half hours after insemination. ca. ×800.

the egg of the trout, reported that the spermatozoon entered the egg with its entire body, but the tail soon after disappeared. The spindle of the egg still persists in the metaphase stage of the second polar division (Fig. 3). At 20 minutes after insemination, the egg-plasm at the part of sperm-entry exhibits remarkable projection. The sperm-head which now completed a rotation of 180 degrees takes a new orientation with its base directing towards the center of the egg. It shows a little swell being slightly larger in size than in the former stage (Fig. 4). The second polar spindle is still arrested at metaphase. Ten minutes afterwards, the projection of the egg-plasm at the part of sperm-entry disappeared, but its site is marked by the occurrence of a very minute protuberance. The sperm-head is found a little more swollen than the former (Fig. 5). From this stage on, the chromosomes of the second polar spindle seem to begin to separate. Then they pass to the opposite poles of the spindle with the passage of time (Fig. 6). About one hour after insemination, a sperm-aster is developed from the sperm-head at its base (Text-fig. 1 and Fig. 7). Behrens (1898), in the egg of the rain-bow trout, described the aster developing in every direction around the sperm-head together with a minute centriole situated at the center of the aster. In the present material also, the centrosome is clearly demonstrated at the central portions of the aster, with a minute centriole. By this time the polar division is found at the telophase stage (Fig. 8). Then, the sperm-head grows larger by swelling with time showing the fully developed astral rays. Fig. 9 indicates the sperm-head with its aster, observed in an egg taken at one and a half hours after insemination. Fig. 10 shows the polar spindle at the same stage, in which the constriction of the polocyte from the egg surface to form the second polocyte is depicted. The sperm-head and the polar spindle observed in an egg taken at about two hours after insemination, are shown in Text-figs. 2 and 3. The sperm-head now grows much larger showing clear zone of cytoplasm around the head; the head still remains in a condensed condition. By this time, the extrusion of the second polocyte is almost completed. At the same time the chromosomes remained in the egg lose their individuality and tend to fuse together into a compact mass of irregular outline. At this time many granular substances staining deeply with haematoxylin are found accumulated in the oöplasm surrounding the sperm-head and egg nucleus. In the eggs preserved at two and a half hours after insemination, th egg nucleus shows many chromosomal elements which scatter along the inner wall of the nucleus with smooth contour (Figs. 12, 13 and Text-fig. 4). With the completion of the egg nucleus into the vacuolized female pronucleus, the sperm-head seems to give off its condensed structure and metamorphose into a vesicular elongated form, with a sudden increase of size, resulting in the formation of the male pronucleus (Figs. 11, 13 and Text-fig. 5). During metamorphosis the male pronucleus advances towards the deeper part of the egg being followed by the sperm-aster to meet the female pronucleus (Fig. 14). The male pronucleus is almost spherical in outline

with a smooth membrane; it measures 0.011 mm in diameter, and is filled with clear, colorless nuclear substance. The male pronucleus at this stage shows the fully developed sperm-aster with remarkable radiation of rays.

At the time of completion, the female pronucleus situated on the egg periphery is elliptical in shape with approximate diameter of 0.014×0.005 mm; it is filled with colorless nuclear substance including minute chromatin granules (Fig. 15 and Text-fig. 6). Then it begins to move towards the deeper part of the egg to conjugate the male pronucleus. Fig. 16 indicates the female pronucleus in the course of migration observed in an egg at three hours after insemination. It is never accompanied by an astral sphere or any like radiating structure. The characteristic feature of the cytoplasm developing ahead and behind the female pronucleus alone serves to indicate the direction of its movement, though it is less pronounced than in the figure presented by Boems (1888). The characteristic



Text-fig. 6. Completed female pronucleus. Notice the second polocyte adhering to the egg by a fine protoplasmic fiber. **Text-figs. 7-8.** Migrating female pronucleus and the male pronucleus at the same stage. From an egg about 3.5 hours after insemination. **Text-fig. 9.** Conjugated pronuclei at the earliest stage. They are still distinguishable from each other by staining capacity and size-difference. From an egg 4 hours after insemination. **Text-fig. 10.** Metaphase of the first cleavage, side view. The chromosomes are separated into two distinct groups. ca. ×800.

structure of cytoplasm ahead the female pronucleus resembles the idiosome described by Makino (1934) in the migrating male pronucleus of *Hynobius*. Fig. 17 shows the male pronucleus in the stage of migration. It is nearly elliptical in form including chromatin elements which are larger and more distinct than in the former stage. The radiation of the astral rays seems to be now less pronounced than in the previous case. Along with the migration, the pronuclei grow in size and the chromatin material in nuclei becomes distinct in appearance. In Text-fig. 7 is shown the female pronucleus during migration which was observed in an egg fixed at $3^1/_2$ hours after insemination. Text-fig. 8 indicates the male pronucleus at the similar stage. The female pronucleus is now as large as the male pronucleus, and shows a weak affinity to stain like the latter. The division of the centrosome is taken place developing the amphiaster between the divided ones; the astral radiation is now less pronounced, though distinct, than that of the previous stage. In Fig. 18 are shown the male and female pronuclei just prior to conjugation. The female pronucleus grows somewhat larger than the male pronucleus. Further the former is readily distinguishable from the latter by the weak affinity of the chromatin elements for stain. The astral system now becomes distinct developing a remarkable radiation. Behrens (1898), concerning the development of the amphiaster from the sperm-aster, stated that "Die Centrosomen der ersten Furchungsspindel leitet Blanc aus der Teilung eines einfachen Centrosoma her, welches vor der Verschmelzung der Vorkerne aus der Vereinigung des männlichen und angebliches beobachtet weiblichen Centrosoma hervorgangen sein soll. Diese Angaben Blanc sind sicherlich irrtumlich. Wie ich oben gezeigt, konnte ich im Forellenei stets nur zwei männlich Centrosomen und keine weibliches find, und eine (Wieder-Vereinigung dieser Centrosomen findet Ueberhaupt nicht statt) ist auch a priori höchest unwahrscheinlich." The findings in the present study are fairly accordant with those of Behrens (1898). There is no evidence for the presence of a female aster so far as the present observations are concerned; it is most probable that the amphiaster originates from the spermaster on the basis of this study.

4) *Movements of the pronuclei and their routes*

It is a remarkable phenomenon in this fish, that the migration of the male pronucleus to the deeper part of the egg is taken place prior to that of the female pronucleus. After the male pronucleus has reached to a certain place, the female pronucleus commences its migration to meet the male pronucleus. A penetration-path of the spermatozoon is nearly vertical to the egg surface and the spermatozoon just penetrated is found lying on the periphery of the egg. Immediately after the penetration, the sperm-head rotates 180 degrees as already noted. About one hour after insemination, the sperm-aster is observed developing near the base of the sperm-head. With the proceeding of the second polar division, the astral radiation grows developing in every direction. Following the extrusion of the

second polocyte the sperm-nucleus begins to migrate to the deeper part of the egg. The sperm-nucleus seems to pass descending vertically from the penetrating point towards the central part of the blastodisc. The penetrating point of the spermatozoon is always found near the top of the animal pole,where the micropyle is present. Therefore, both the penetration-path and the copulation-path of the spermatozoon constitute a continuously straight line, without the formation of remarkable angle by the two paths. In amphibian eggs, the two paths generally form a remarkable angle in this case. The movement of the sperm-nucleus occurs very quickly ; its migration commences at about two and a half hours after insemination and it is completed in a period of about thirty minutes. During the migration, the sperm-aster develops its radiation, and the head of the spermatozoon is converted into the male pronucleus.

The second polar spindle is formed at a little distance from the top of the animal pole. After the extrusion of the second polocyte, the chromosomes contained in the egg reconstruct the nucleus developing into the female pronucleus, and the formation of the female pronuclus has been taken place with the migration of the sperm-nucleus. On arrival of the male pronucleus at the meeting point, the female pronucleus begins its movement. The female pronucleus moves also quickly ; its migration occurs at about three hours after insemination and is generally finished in a period of about thirty minutes. The course of movement of the pronucleus is marked by a track as described above, though it is not so remarkable as observed in amphibian eggs. The path apparently traces a parabola. The radiation becomes gradually inconspicuous with the approach of the two pronuclei. After the conjugation the pronuclei are persisted in its place throughout the whole period in preparation for the first cleavage.

The causes that determine the movements of the pronuclei during fertilization of the egg are now unknown, though there are some assumptions presented by earlier workers as follows : the two pronuclei approach each other by means of amoeboid movement (Fick 1893), or approach and union of the nuclei are determined by some kind of attraction between them (Wilson 1900), or they are passively drawn together by the rays of the sperm-aster or by protoplasmic current in the oöplasm (Conklin 1899). On the basis of the present study it seems most probable that the sperm-aster takes an important role in the movement of pronuclei, though not definitevely stated at present.

5) *Conjugation of the pronuclei and the formation of the first cleavage spindle*

Generally at about 4 hours after insemination, the male and female pronuclei come in contact. In the first place, they are touched at a part of their bodies, then they come to lie side by side with the nuclear membrane in intimate contact. But no actual fusion of them occurs at all ; in this condition they remain throughout the whole period in preparation for the first cleavage. In the eggs preserved at $3^1/_2$ hours after insemination, the conjugation of the pronuclei has met

with in five of twelve cases examined. About 4 hours after insemination, all eggs are found completed in the copulation of both pronuclei. From this fact it is most probable that the meeting of the pronuclei generally occurs within 3 to 4 hours after insemination has taken place. The position at which the two pronuclei meet is never at the geometric center of the blastodisc, but approaches a little towards the upper pole. The pronuclei just after conjugation are still distinguishable from each other by their staining capacity and also by the size-difference (Text-fig. 9). At the time of conjugation the male pronucleus is smaller in size than the female pronucleus. The chromatin elements of the male pronucleus is more distinct than those of the female pronucleus. In the next stage, two centrosomes after division migrate oppositely and finally take positions on opposite sides of the conjugating plane of the nuclei. The astral system displays its remarkable radiation. The pronuclei in contact are now apparently alike in their structure, size and other nature, so that it is usually impossible to distinguish them with certainty. The chromatin elements of the nuclei become more distinct and better defined by taking stains than in the former stage (Fig. 19). In the eggs preserved at about 8 hours after insemination, the conjugation-nuclei are seen much elongated and the osculating plane attains a great dimension. But they are still distinctly separated by the nuclear membrane (Fig. 20). There are a certain number of chromosomal elements of uniform thickness in both nuclei. Shortly later, the nuclear membrane disappears and the formation of the spindle is formed at the metaphase stage of the first cleavage (Fig. 21). Thus there is no actual fusion of the two pronuclei throughout the whole course of the prophase of the first cleavage, but they remain in close contact being separated by nuclear membrane. In the fully formed metaphase figure two different groups of chromosomes, paternal and maternal in their origin, still remain distinctly separated as is most clearly recognizable in lateral aspect (Fig. 22 and Text-fig. 10). At anaphase, each group of chromosomes has divided into equal halves respectively. The evidence here presented is in fair accordance with those clearly demonstrated already in *Hynobius* and *Mus* by Makino (1934, 1941) and in the carp by Ozima (1943). Generally the first cleavage spindle has been found in the process of division in the egg fixed about 8 hours or some more after insemination.

Discussion

Wilson (1928) classified the mode of fertilization into two main extreme types with respect to the behavior of the male and female pronuclei after conjugation in the egg ; the one is the sea-urchin type and the other the *Ascaris* type. In the sea-urchin type the pronuclei conjugate immediately after entrance of the sperm and apparently fuse completely to form a fusion-nucleus. In the *Ascaris*-type, on the other hand, the sperm enters the egg before the polar divisions of the egg have been accomplished, and after conjugation of the pronuclei no direct

mingling of contents occurs between them in spite of intimate contact. There is no question based on the results of the present study and those established by some earlier workers in the fact that in the salmon and trout eggs the entry of the sperm takes place in the metaphase stage of the second polar spindle after the first polocyte has been extruded from the egg, and therefore, the sperm-nucleus resides *in situ* within the egg until the polar division has been accomplished. During this pause the spermatozoon is converted into the male pronucleus. Then the male pronucleus grows larger and becomes almost indistinguishable from the female pronucleus. Thus the two pronuclei are nearly identical in size at the time of union, but they are still distinguishable on account of the staining capacity of the chromatin elements included in them. The centrosome with its astral rays divides into two developing the amphiaster between them, before karyogamy takes place. In this respect the results of observations of Blanc (1894) are doubtful in describing that the amphiaster of the first cleavage spindle originates from the centrosomes of a fusion-nucleus. In the salmon eggs, Blanc (1894) observed the actual fusion of two pronuclei and described as follows "9.5 hours after insemination, the structual contents of the female pronucleus mingle with that of the male pronucleus to form a homogeneous cleavage-nucleus. At the same time the astral rays completely disappear." On the contrary, Kupfer (1886) and Behrens (1898) reported that the male and female pronuclei do not actually fuse after coming in contact, but remain side by side with nuclear membrane intact. And recently Ozima (1943) reached the similar conclusion in the study of the carp. Still further, the results of the present investigation confirmed beyond question the phenomenon in showing that the male and female pronuclei after conjugation remain separated throughout the whole preparing stage for the first cleavage with nuclear membrane intact, and further that at the time of cleavage the chromosomes remain again separated into two distinct groups of paternal and maternal origin. Viewed from the reported case in various forms of vertebrates, it can be said with certainty that the egg of fish belongs to the *Ascaris* type in the mode of karyogamy[1].

The noticeable features of the egg of the salmon at the time of fertilization concern the metamorphosis of the spermatozoon after insemination and its behavior in migration. In amphibian eggs, the spermatozoon, after entrance into the egg, shows a complete metamorphosis into the male pronucleus of a vacuolated form during a period in which the polar division has been accomplished (King 1901, Makino 1934), while in eggs of the salmon the sperm-head remains in a compact chromatic condition without metamorphosing into a vacuolized nucleus. In this case the sperm-head completes its metamorphosis into the male pronucleus after the extrusion of the second polocyte. Recently R. Chambers

1) Details on this respect, refer to Makino (1934, 1941).

and E. L. Chambers (1949) have studied on the nuclear and cytoplasmic inter-relation at the time of fertilization in the *Asterias* egg which develops in sea water commencing with germinal vesicle stage and can be inseminated at any time ; they observed that the maturation of the karyocytoplasm[1] tends to lead directly to the formation of two polar bodies and the maturation is completed at about the time of first polar body formation, and that the rate at which the fertilization events proceed depends upon the cytoplasmic maturation and immature state of the karyocytoplasm has a delaying effect on the development of the sperm and its accompanying events. With the above view in mind, it is highly probable that the condition of karyocytoplasm in the salmon egg at fertilization is not equivalent to those of amphibian or other vertebrate eggs and the former is more immature than the latter, since in the salmon egg the sperm-head remains not metamorphosed after entrance into the egg until the extrusion of the second polocyte has accomplished, though the salmon eggs show, when laid, the second metaphase spindle observed in other eggs. This is of importance with reference to the following evidence that the salmon eggs are spontaneously activated by water without insemination, accompanying the extrusion of the second polocyte, thus, insemination never takes an important role for the formation of second polar body as in the case of *Asterias* (Yamamoto 1951), while in amphibian egg, stages subsequent to the metaphase of the second division take place only after entry of the spermatozoon into the egg.

The other remarkable feature of the salmon eggs points to the migrating behavior of the pronuclei. In the eggs of amphibia as in the case of other animals, both pronuclei start at the same time to migrate towards the meeting place. But in the salmon eggs the migration of the male pronucleus has been taken place prior to that of the female pronucleus. After the migration of the male pronucleus has reached the meeting point, the female pronucleus commences its migration. The causes that determine the movements of the pronuclei during fertilization are left not answered ; they are the important items in fertilization which demand further research in future.

Summary

1) At the time of insemination the second maturation division of the egg persists in the stage of metaphase and then it gradually advances in further course of division after entry of the spermatozoon.

2) The region where the spermatozoon has entered is found marked by a funnel-shaped depression, assuming a feature like the so-called "entrance cone." About ten minutes later, the entrance-region is found as a tiny projecting area

1) By Chambers and Chambers (1949) the cytoplasm of the maturing and mature egg was termed karyocytoplasm.

at the surface of the egg. The projecting area of the oöplasm disappears after a while.

3) About one hour after insemination, a sperm-aster is apparent lying at the base of the sperm-head. The polar division in the egg has found proceeded to the telophase stage by this time.

4) Following the extrusion of the second polocyte, the chromosome mass in the egg begins to convert into the vesicular female pronucleus. Together with the metamorphosis of the egg-nuclus, the sperm-head of the compact chromatic condition becomes vesicular showing less affinity for stains. Along with these changes the sperm-head advances towards the deeper part of the egg accompanying the sperm-aster.

5) About three hours after insemination, there is found the metamorphosed female pronucleus resident at its original position together with the sperm-head which is completely converted into the vesicular male pronucleus. At this stage the male and female pronuclei are apparently alike in structure, but the former is readily distinguishable from the latter on account of the occurrence of the sperm-aster. There is no evidence for the presence of radial system in the female pronucleus.

6) After metamorphosis, the female pronucleus commences its migration to approach the male pronucleus which at that time has already arrived at the meeting position. The meeting of the pronuclei generally takes place within 3.5 to 4 hours after insemination has occurred.

7) At the time of conjugation the pronuclei are still distinguishable from each other due to both staining capacity and size-difference. The male pronucleus is characterized by showing more distinct chromatin elements than the female pronucleus. The male pronucleus is generally smaller in size than the female pronucleus.

8) After the pronuclei come in contact, they do not actually fuse, but lie side by side in close contact with the nuclear membrane intact. Thus the maternal and paternal chromosome material are separated in distinct groups during through the stage prior to the first division, and further the chromosomes remain again separated into two groups of the maternal and paternal origin at the time of cleavage.

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Explanation of Plate V

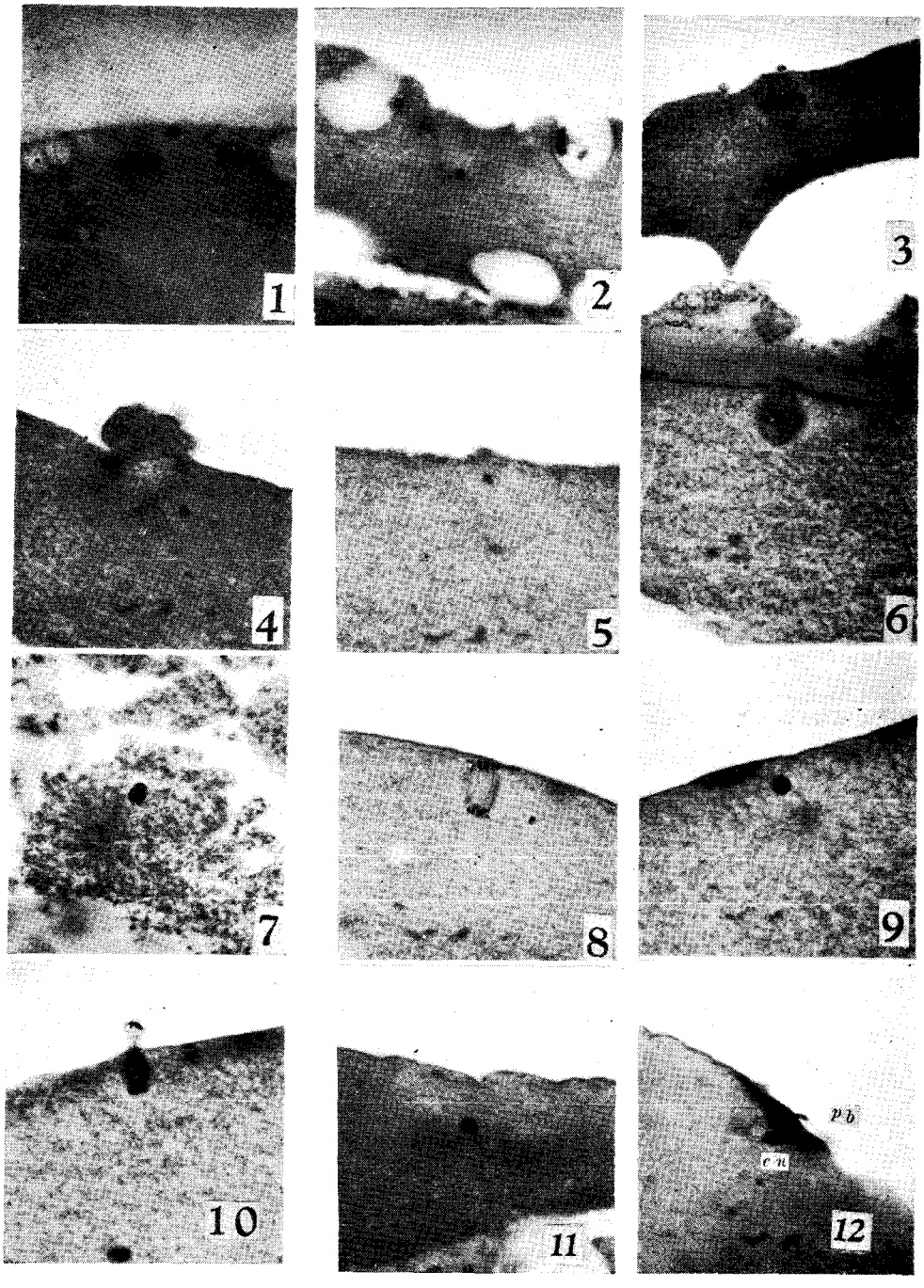
All figures are photomicrographs of sections of the eggs.

Fig. 1. Sections of an egg directly striped out from a mother fish, showing the second polar division metaphase, oblique view. ca. ×650. **Fig. 2.** "entrance cone" and sperm-head. From an egg about 10 minutes after insemination. ca. ×600. **Fig. 3.** Metaphase stage of the second polar division, side view. From an egg at same stage as above. ca. ×600. **Fig. 4.** Sperm-head and projecting cytoplasm at the region where the spermatozoon has penetrated. Sections from an egg about 20 minutes after insemination. ca. ×600. **Fig. 5.** Sperm-head about 30 minutes after insemination ca. ×600. **Fig. 6.** Early anaphase stage of the second division. The same as Fig. 5. ca. ×600. **Fig. 7.** Sperm-head and sperm-aster about 60 minutes after insemination. ca. ×600. **Fig. 8.** Telophase stage of the second polar division. The same as fig. 7. ca. ×600. **Fig. 9.** Swollen sperm-head about one and a half hours after insemination. ca. 600. **Fig. 10.** Sections of the eggs of same stage as above. The second polocyte is about to constrict off, in which the clumping of the chromosomes takes place. ca. ×600. **Fig. 11.** Sperm-head beginning the process of metamorphosis. From an egg about two and a half hours after insemination. ca. ×600. **Fig. 12.** Condensed mass of sister chromosomes remained in the egg after the second polocyte has been extruded. The same as Fig. 11. ca. ×600 . e.n., egg nucleus. p.b., second polar body.

Explanation of Plate VI

All are photomicrographs of sections of eggs.

Fig. 13. Metamorphosing sperm-head and egg nucleus about two and a half hours after insemination. The sperm-head has already migrated to the inner art of the egg. ca. ×600. f, female nucleus, m, male nucleus. **Figs. 14-15.** Already metamorphosed male and female pronuclei. From an egg about three hours after insemination. ca. ×600. f.n., female pronucleus. m.n., male pronucleus. p.b., polar body. **Fig. 16.** Migrating female pronucleus three hours after insemination. ca. ×600. **Fig. 17.** Male pronucleus at same stage as Fig. 16. ca ×600. **Fig. 18.** Two pronuclei just prior to conjugation. From an egg about $3^1/_2$ hours after insemination. ca. ×600. **Figs. 19-20.** Conjugated nuclei., preparing for the first cleavage mitosis. Fig. 19 and 20, from the eggs about 6 and 8 hours after insemination respectively. ca. ×600. **Fig. 21.** First cleavage spindle metaphase. From an egg about 8 hours after insemination. ca. ×600. **Fig. 22.** Metaphase stage of the first cleavage. The chromosomes are segregated into two distinct groups, probably of paternal and maternal origin respectively. From an egg about 9 hours after insemination. ca. ×600.



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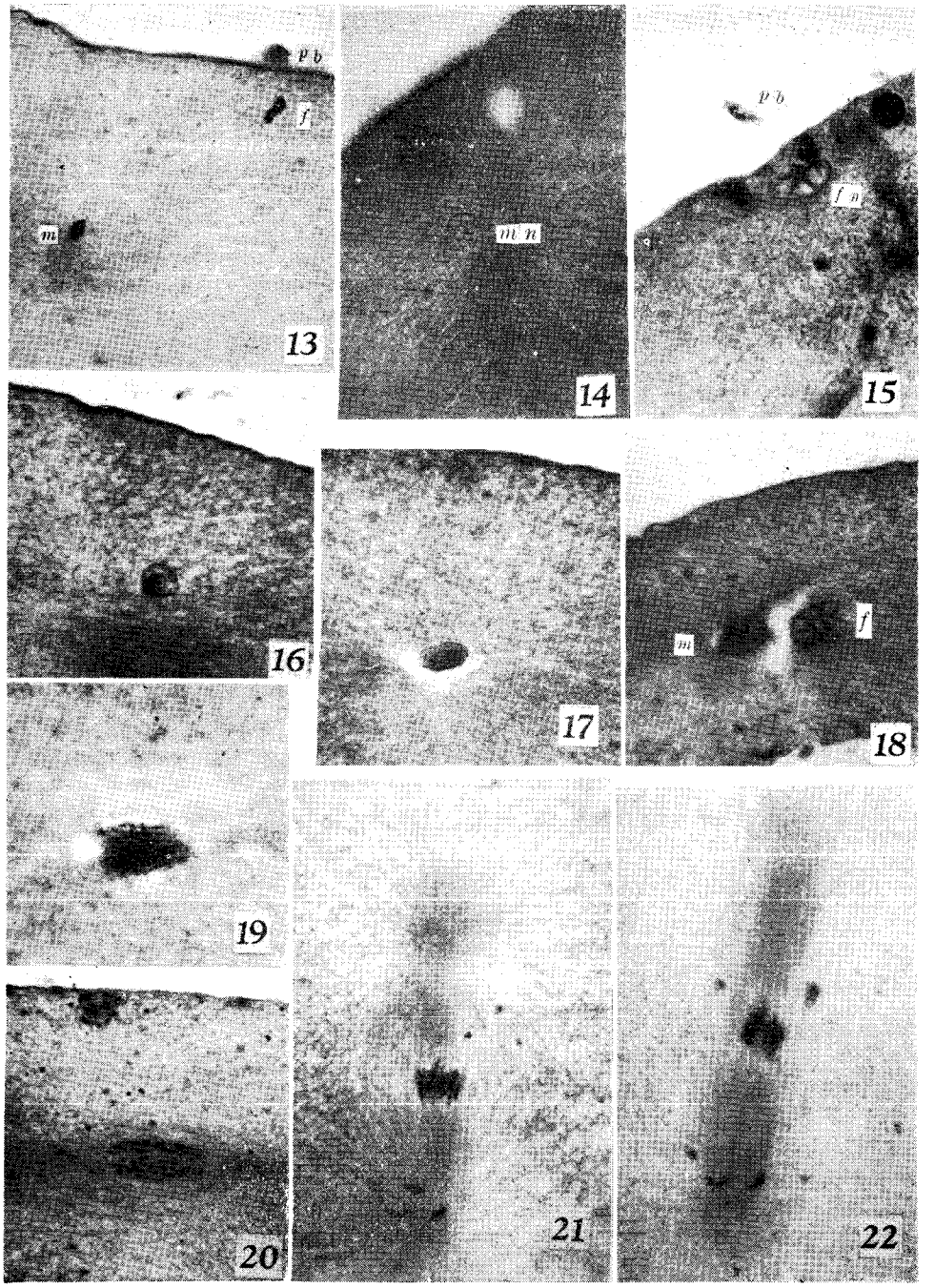

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